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II. *Catalase Test For Mastitis

** *Keith T. Maddy, D.V.M.*

INFECTIOUS BOVINE MASTITIS is one of the major diseases plaguing the livestock industry today. Although there are several good therapeutic agents for eliminating many of the infectious organisms from cows' udders, a major problem which still remains is a quick, accurate, easy, on the spot test to determine the presence or absence of mastitis.

Direct bacteriological cultural examination of the milk is the most accurate of all the diagnostic tests, but it is very tedious, time consuming, and so involved that most veterinarians will not perform it themselves over a long period of time. When the tests are made by a laboratory there is sometimes more of a time loss between test and treatment than the herd owner wants. Also, since some of the new combined antibiotics are fairly effective in eliminating many of the infections present, the diagnosis of a specific etiological bacteria is not always considered as important as merely diagnosing the presence of an infection.

Indirect methods of diagnosis of mastitis are generally much quicker than the cultural methods; however, they are usually more inaccurate. About the most accurate of the indirect tests is the catalase test. Although this is a relatively easy test to use, it is used surprisingly infrequently in practice.

The first studies on catalase in milk were made almost fifty years ago by such men as Loew[4], Koning[3], Gratz[1] and Naray[7]. Catalase is a heme compound which is a constituent of cytochrome and hemoglobin. It aids in the transfer of oxygen in plant and animal cells and is bound to erythrocytes, leucocytes, platelets and epithelial cells in milk. In cases of mastitis the leucocytes in the milk increase markedly in number, thus increasing the catalase content. The other cellular elements increase also but not nearly as much as the leucocytes.

It can be seen that a leucocyte count of milk samples is a valuable indirect test for the presence of mastitis. However, investigators are not decided on just where to draw the line between milk from a normal quarter and an inflamed quarter. Some set the figure as low as 100,000 leucocytes per ml. and others as high as 1,000,000 per ml. Merchant and Packer[6] state that a count of 500,000 per ml. usually indicates an infection if cows early and late in lactation are excluded. McFarlane, Blackburn, Malcom and Wilson[5] in a study of mastitis point out that a persistent cell count of 100,000 per ml. indicates inflammatory changes. They conclude that over half of the early cases of mastitis

* This is the second in a series of three articles written on Bovine Mastitis by Dr. Maddy. Article III, dealing with Penicillin Treatment of Mastitis, will follow in a subsequent issue.

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escape detection if diagnosis is based solely on laboratory cultural tests, but only five percent of these cases are missed if the cell count test is used. However, they point out that a high leucocyte count on milk on antemortem examination and the presence of mastitis as evidenced by postmortem histological examination can not always be correlated with the finding of the typical mastitis organisms, this indicating that other, yet unknown etiological factors may be operative in the early stages of mastitis.

The Lind apparatus for catalase determination was developed by Orla-Jensen and Lind[8]. It consists of a 20 ml. test tube with a one-holed rubber stopper with a piece of S-shaped glass tubing inserted in it. Merchant and Packer[6], and Halverson, Cherrington, and Hansen[2] have reported on the use of the Lind apparatus for mastitis diagnosis.

The test consists of placing 15 ml. of milk in the tube and adding five ml. of a solution of hydrogen peroxide, varying from one to three percent in strength. The stopper is inserted, the tube inverted and held at a temperature varying from 75 to 100°F. for from three to six hours. The catalase reacts with the hydrogen peroxide and free oxygen and water are produced as follows: $2H_2O_2 + \text{Catalase} \rightarrow 2H_2O + O_2 \uparrow$. The free oxygen fills the upper end of the inverted tube and the amount present can be measured to determine the amount of catalase present in the milk. Volumes more than 1.5 to 2.5 ml. of oxygen are considered abnormal.

The catalase test rarely misses a case of infectious mastitis unless the infection is almost completely walled off. Monlux[7] points out that there are times when the test gives a positive reaction for excess catalase which is not due to infectious mastitis. They are due to (1) milk being tested during a cow's first four weeks of lactation; (2) to non-infectious mastitis; (3) to milk coming from a quarter low in production; (4) to the possibility of a rise in the catalase content of milk when a cow is in estrus; and (5) to the fact that there may be excess catalase for as long as 30 to 40 days after antibiotic treatment of an infected quarter. At first glance it might appear that the test has many shortcomings; however, in actual practice they are quite minimal. The herdsman can generally point out to the person interpreting the test the cows that are early in production, low in production, those that have been treated in the last 40 days and those in estrus. Furthermore, if individual quarter samples have been taken, these interfering factors increase the catalase content of each quarter equally (except in the case of a recently treated quarter), and a case of mastitis increases the content of the affected quarter even more. This is evident when the tests are interpreted.

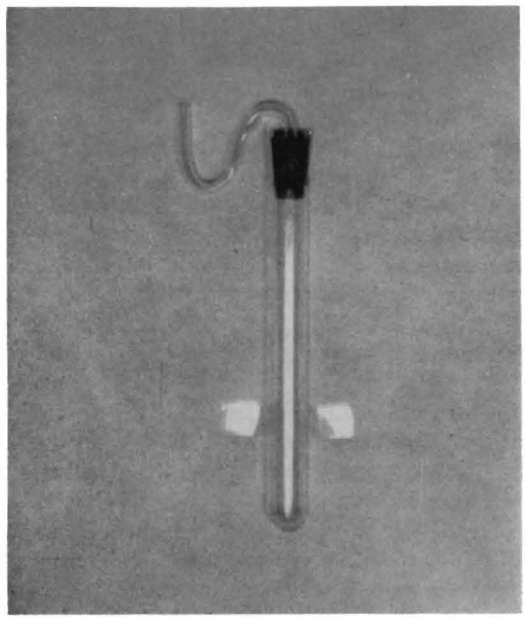

Fig. 1. The Lind Apparatus.

It can be seen that the catalase test is a good test for mastitis for it is uncommon for it to miss a case of mastitis. (Even cultural tests often miss the same cases because of the small number of infective bacteria present at the time of the test.) When the test is intelligently interpreted the only completely undetectable false positive is in the case of non-infectious mastitis. (In actual practice a case of non-infectious mastitis often receives internal antibiotic treatment anyway because these cases become infected at this time if exposed to the slightest infection. The cost of treatment is so low compared

to the chance of infection that the risk is not worth it if the cow is of much value.)

Test Procedure

The test as described by Merchant and Packer[6] is performed as follows:

1. Pour 5 ml. of one percent H_2O_2 into a 20 ml. test tube.
2. Add 15 ml. of freshly drawn milk.
3. Stopper immediately with a one-hole rubber stopper containing a piece of S-shaped glass tubing.
4. Invert the tube and place on a rack so that the closed end of the test tube is up.
5. Incubate at 37°C. (98.7°F.) for three hours.
6. At the end of the time, measure the amount of oxygen present in the tubes.

Interpretation

1. Normal milk gives about 1.0 ml. of oxygen.
2. Chronic mastitis gives about 1.5 to 10 ml. of oxygen.
3. Acute mastitis gives more than 10 ml. of oxygen.

Personal Experiences

Over a two-year period and while in a dairy cattle practice, the author performed thousands of catalase tests and found it quite satisfactory. For an incubator a box two feet square with a hinged lid was built, with a light bulb inside for a heat source. In a room of about 70°F. a 75-watt bulb was just about right to hold the incubator at 98 to 100°F. When the outside temperature would markedly affect the incubator, the size of the light bulb was varied.

When this test was first used, the author was of the impression that the samples had to be incubated at just 98-100°F., a critical range. However, in practice this did not prove to be true. Once, due to a power failure, the light bulb went off a few minutes after the tubes had been placed in the incubator. On returning three hours later the power was still off, but surprisingly, the catalase reaction was

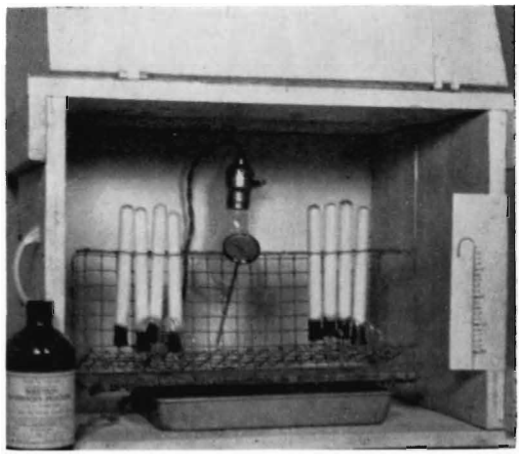

Fig. 2. Milk and hydrogen peroxide in tubes ready for the reaction to begin.

proceeding at room temperature (73°F.) although at a slower pace. At the end of six hours the tests appeared as a typical set. After this, 221 duplicate sets of tests were carried out — one set was placed in the incubator at 100°F. for three hours and the other set was left at room temperature for six hours. The results were almost identical. This knowledge was useful when tests were conducted on a farm without electricity.

The amount of catalase in the milk was found to increase during the heat period. This fact was brought to light during the testing of cows participating in an artificial insemination program as well as in the mastitis control program. On several occasions when a cow was tested on the same day she was inseminated, she gave a positive catalase test in all four quarters of about equal amounts. It was soon decided that the heat period increased the catalase content of milk; after that, cows in heat were especially noted when a herd test was made. An average of the catalase values of 42 cows' milk tested while in estrus was 2.1 ml. This included the normal milk catalase value of about 1 ml.; thus 1.1 ml. on the average was due to the heat period. The increased catalase values were noticed the first milking after the heat period began; they were at their height 24 hours later; and by 48 hours they were usually back to normal. The catalase values for each quarter were generally always quite close together as to

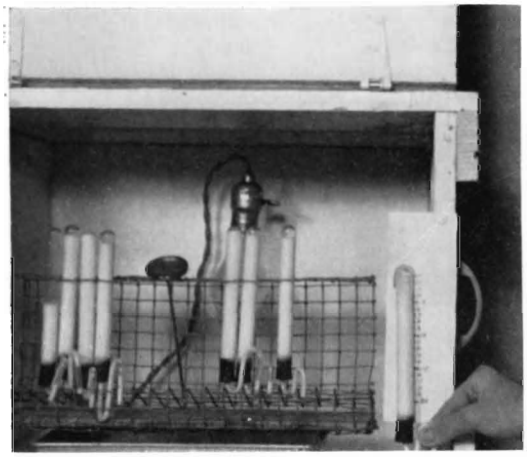

Fig. 3. Measuring the amount of gas. This case released four ml. of gas and represents a chronic case.

amount. If mastitis was present, the affected quarter had an additional catalase value. The quarters with questionable tests were checked by Hotis tests and microscopic examinations.

The accuracy of the catalase test did not vary when dealing with different types of infective organisms except it was more sensitive in diagnosing slight *Staph. aureus* and *Coryn. pyogenes* infections, no doubt due to the pyogenic nature of these organisms.

The direct microscopic examination of an incubated sample is the most accurate of the tests, especially if followed by culturing. However, it is so tedious it was considered impractical on a large scale. The catalase test, correctly interpreted, (considering its limitations as to false positives) was in fairly close agreement with the finding of infective bacteria by the examination of a milk smear by the microscopic method and was in even closer agreement with the leucocyte count of the incubated sample.

Because of the close agreement of the catalase test with the direct tests, after the first 500 cows were tested, the author discontinued most all other routine testing and concentrated on the catalase test. Other tests were performed only when there was definite doubt as to the proper interpretation of the catalase test or when the percentages of the various bacteria infecting a herd were to be determined.

One caution is in order for those who use the test: Be sure that the hydrogen peroxide is fresh and has not lost its strength. If the solution is weak the oxygen production will be below normal.

Summary

Advantages of the catalase test for mastitis:

1. It is the most accurate of the indirect tests.
2. It is the quickest test with a high degree of accuracy. (Treatment can be started in three hours.)
3. It rarely misses cases of mastitis.
4. False positive tests can be determined if accurate information is obtained from the herdsman.
5. It is inexpensive.
6. Equipment is easy to wash, as milk has not soured and stuck to the tubes.

Disadvantages of the test:

1. Some false positives tests can be misleading. (However, in actual practice if there is any doubt about a test, the quarter is usually treated anyway because the cost of the treatment is so insignificant compared to the potential harm an infection could produce that most all herd owners ask for it.)
2. In spite of the fact that the author considers the test the best there is for a general practitioner to use, there is yet a definite need for an accurate instanteous mastitis test. Udall[9] describes a plate test using six to nine percent hydrogen peroxide on a drop of milk. Bubbles are produced in positive cases. This test was used but found only easy to interpret in acute cases—the line between the gas produced by normal milk and that produced by chronic cases could only be guessed at.

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Hibernating animals have a prolonged clotting time. For example, an active squirrel has a normal blood coagulation time of eight minutes, while the hibernating squirrel requires over 49 minutes to provide a partial clot; in fact, a firm clot never forms. The purpose of this action is evident since the blood circulation slows up greatly during the inactive season. It is apparent that lesser clotting properties are necessary when the flow is so greatly reduced.

GAS-TREATED SILAGE

Gas-treated grass for silage was announced by the Ansul Chemical Company at the Chemical Industries Exposition. Research director C. V. Mars said that cattle find the gas-treated silage palatable; surprising, because his new method consists of treating fresh-cut grass with liquid sulfur dioxide, which gives the odor to rotten eggs. Other silage preservatives depend upon fermentation to stop bacterial action, but sulfur dioxide stops fermentation by the weak acid formed when it combines with the moisture in the grass. Studies made at Pennsylvania State College are said to show savings of up to $2 a ton for silage treated with sulfur dioxide. The chemical is shot into the ensiled crop with a copper lance at two-ft. intervals, at depths of one to five ft. until the entire crop is saturated. About five to six lbs. of chemical are required to treat a ton.